THE CULTIVATION OF BACTERIUM ABORTUS BANG*

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Many methods have been described for the cultivation of Bact. abortus Bang. Following the original work with this organism by Bang and Stribolt in 1897, several investigators have described methods for the cultivation of this germ. The most frequently mentioned and used are those of Nowak, Holth, Priez and Fabyan. Recently Stafseth [1] and Huddleson [2] have described culture mediums and methods of growing Bact. abortus which differ from those already in use. The former recommends a medium prepared from liver and spleen. He states that "Strains of the abortion bacillus have been isolated more easily by the aid of these media." A glass jar from which the air was partially exhausted by a suction pump was used in which to grow the cultures. Huddleson emphasizes the importance of an increased carbon dioxide tension for growing Bact. abortus Bang. His conclusions are:

There is sufficient proof that:

(1) The growth of Bact. abortus is not due to a reduced oxygen tension.

(2) A carbon dioxide tension greater than that of the air governs and greatly facilitates the primary growth of Bact. abortus.

(3) An atmosphere containing (by volume) 10 per cent. of CO_2 gas appears to produce the earliest and most luxuriant growth of Bact. abortus.

Huddleson recommends the use of a generator containing calcium carbonate to which hydrochloric acid is added as a source of the CO_2.

We have been working with Bact. abortus for many years and have experienced the same difficulty as other authors in isolating the organism. All the methods mentioned have been used with more or less success. The abortion germ usually grows with great difficulty in the cultures made from the original material as the stomach contents of an aborted fetus. After two or three transfers, the bacilli grow quite readily even under ordinary aerobic conditions.

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[1] Studies in Infectious Abortion, 1922.

[2] Technical Bulletin 49, Mich. Agric. College, Exper. Station, 1921. Cornell Veterinarian, 1921, 11, p. 210.

The method of sealing the inoculated tubes with paraffin, originally described by Holth, has given us fairly uniform results. Sealing wax has also been employed for closing the tubes. Various kinds of medium have been inoculated and the resulting growths compared. It is not considered necessary to give the details of the numerous experiments and therefore only the results are stated.

Following the report of Huddleson the CO_2 method was employed. Certain difficulties were encountered. The gas given off by the generator described by this author contained a considerable amount of chlorin which had an inhibiting effect on the growth of the abortion germs. Also, the generator is not convenient to use, because of the short time during which the reaction goes on and during which the gas is given off. We have used in place of the generator, a cylinder of commercial CO_2 with much greater success. The medium which seems to give us the best results is beef infusion agar. This should be made from lean beef and 2% agar added. It should be adjusted to a P_H of 6.8 to 7.2. We have found a slightly more alkaline medium to be better for the growth of Bact. abortus Bang than heretofore reported. At the time this medium is to be used, approximately 10% naturally sterile horse serum should be added to the melted agar, cooled to 50 C., and the tubes allowed to solidfy in a slanting position.

These tubes are heavily seeded with the material to be cultivated and then placed in a round Withal Tatum museum jar, the capacity of which is known. The ordinary rubber washer furnished with these jars will not last, as they soon press out. Reinforced rubber diaphragm sheet packing $\frac{3}{16}$ in. thick has been used continually for more than a year with good results. The washer made from this material should be liberally smeared with lanum (Merck) in order to make an air tight connection between the top of the jar and cover.

The inoculated tubes are placed in the jar and with the cover raised only sufficiently to allow the rubber tube (8, fig. 1) from the carbon dioxide tank (1, fig. 1) to enter and approximately 10% of the gas added. The rubber tube is removed quickly, the cover screwed down tightly and placed at 37.5 C. After 24 hours' incubation small pin point colonies will be noted on the medium by observation through the glass walls of the jar. Forty-eight hours' incubation shows well developed colonies of Bact. abortus.

Huddleson states that carbon dioxide is directly responsible for the acceleration of the growth of Bact. abortus. Up to the time of the publi-

cation of this work it was believed that reduced oxygen tension was the particular factor necessary for the development of this germ. In order to test the specificity of carbon dioxide, duplicate cultures were made and placed in a jar containing 10% hydrogen and others in 10% carbon dioxide. The two groups of cultures were compared at periods of 24, 48 and 72 hours. No difference in the vigor of the

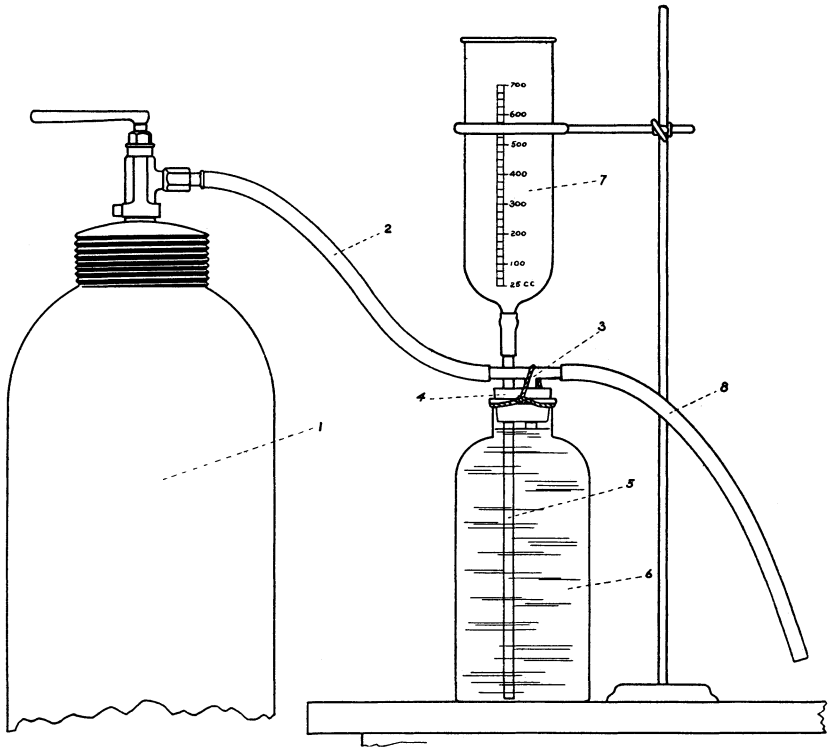

Fig. 1.—Apparatus for the addition of carbon dioxide or hydrogen for the cultivation of Bact. abortus Bang; 1, gas tank; 2, rubber tube; 3, glass T tube; 4, two hole rubber stopper (tied in); 5, glass tube leading to bottom of bottle; 6, bottle filled with water; 7, graduated open receptacle for measuring amount of water displaced by the gas; 8, tube leading to the jar in which the cultures are placed.

growth or the microscopic appearance of the organism was noted. It therefore does not seem likely that carbon dioxide exerts any specific action on the growth of Bact. abortus. This was also suggested by Edwards [3] in his review of Huddleson's article. He states "Beyond showing that an atmosphere in which a certain rather high percentage

[3] Vet. Record, 1922, 2, p. 31.

of the gases normally present has been displaced by CO_2 is favorable for the growth of B. abortus, Huddleson does not appear to have proved that this gas has any specific properties."

CONCLUSIONS

Horse serum beef infusion agar with a P_H 6.8-7.2 is excellent for cultivating Bact. abortus Bang.

The serum agar cultures develop rapidly in an atmosphere of 10% carbon dioxide and hydrogen.

This method of growing Bact. abortus has given us our most uniform and satisfactory results.